Short note

Biochemical and physiological adjustments in common Bermudagrass (*Cynodon dactylon* [L.] Pers.) and tall Fescue (*Festuca arundinacea* Schreb.) under low temperature stress

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Abstract: Low temperature is a restrictive factor for turfgrass growth and development in temperate regions. A study was conducted with the purpose of examining the physiological and antioxidant response of two turf species, *Festuca arundinacea* Schreb. 'Starlett' and *Cynodon dactylon* [L.] Pers. 'California Origin' to cold stress in a growth chamber at the College of Agriculture, Shiraz University. Five temperatures (25, 15, 7.5, 0 and -7.5°C) in four replicates were examined in a completely randomized design experiment. It was revealed that under low temperature stress, soluble sugar contents, proline, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were increased in both turfgrasses. Antioxidant enzyme activity, particularly catalase (CAT, EC 1.11.1.6) and superoxide dismutase (SOD, EC 1.15.1.1), was increased as a result of temperature reduction from 25°C to 0°C. Tall fescue is thought to be better adapted to cold stress than common bermudagrass due to higher soluble sugar contents, proline, malondialdehyde and antioxidant enzyme activity. The results show that scavenging enzymes have a direct effect in cold season tolerance of turfgrass and improve the defense mechanism of plants, but their exact role merits further investigation.

1. Introduction

Temperature is a limiting element for distribution of plants worldwide (Sakai and Larcher, 1987). Cold season turfgrasses such as *Festuca* in temperate regions have a good adaptability to low temperature, but under severe winter conditions may suffer considerable damage (Levitt, 1980). Although fescues are cultivated in transition zones, warm season turfgrasses such as bermudagrass are preferred (Carrow, 1994). Plant responses to cold stress and subsequent adaptation occurs at physiological and biochemical levels, as well as cellular and molecular extents (Gulzar *et al.*, 2011). Harsh low temperatures result in oxidative stress and a change in proline and sugar content of the cells. The vital means for interaction of plants to these stresses is a balance between antioxidant enzymes and reactive oxygen species (ROS). The damaging ROS responsible for oxidative stress consists of free radicals: superoxide ($O_2^{.-}$), hydroxyl ($OH^.$), hydroperoxyl ($HO_2^.$) and other molecules such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Gill and Tuteja, 2010). The precise role of antioxidant enzymes which give tolerance to cold stress in turfgrasses has not yet been in-

vestigated, but their relieving effect to other oxidative stresses has been reported by other researchers (Jiang *et al.*, 2005). Rogers *et al.* (1975) examined the proline amount of *Zoysia japonica* Steud. 'Meyer' during the months from October to March and found that there is an increase in proline from October to December. It has been shown that for the period of adaptation to cold, SOD and CAT activity in *Agrostis stolonifera* L., *Poa pratensis* L. and *Lolium perenne* L. significantly increased (Sarkar and Bhowmik, 2009).

The main objective of the present study was to investigate the effects of low temperature stress on biochemical and physiological responses of tall fescue and common bermudagrass. To the best of our knowledge, this is the first report on how these turfgrasses counter cold stress.

2. Materials and Methods

Plant materials and experimental conditions

The experiment was conducted in a growth chamber (Gallenkamp, Germany) at the Department of Horticultural Sciences at the College of Agriculture, Shiraz University (29°36' N and 52°32' E, elevation 1810 m). Seeds of common bermudagrass (*Cynodon dactylon* [L.] Pers. 'California origin') and tall fescue (*Festuca arundinacea* Schreb. 'Starlett') were cultivated in 5 L plastic pots containing a

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mixture of 1:2 (v/v) of loamy soil/decomposed farmyard manure. Irrigation was carried out on a daily schedule. Established turfs were clipped from 3 cm above ground by a hand mower and were transferred to the growth chambers prior to the application of treatments. All treatments received a constant light intensity of 3000 Lux, relative humidity of 65±5% and a 12 h photoperiod. Low temperatures were maintained at 25, 15, 7.5, 0 and -7.5°C for 48 h.

Experimental design and data analysis

Experiment factors were arranged in a completely randomized design with four replications. Data were analyzed using SAS software (ver. 9.1.3) and means were compared using the least significant difference (LSD) test at p<0.05.

Reducing sugars and proline content

Phenol-sulfuric acid reactions were used to determine the reducing sugar content. Shoot samples were oven dried at 60°C for 48 h and then ground to a fine powder using an electric mill. Samples (0.2 g) were diluted with 80% ethanol and centrifuged at 13500 rpm. Supernatant was further diluted to 25 ml by 80% ethanol. Then, 1 ml of extract was mixed with 1 ml of 5% phenol. Five ml of concentrated sulfuric acid were added to tubes and immediately stirred. Light absorption was measured by a spectrophotometer (Biochrome, UK) at 490 nm wavelength (Dubois *et al.*, 1956). Proline was determined according to the method used by Bates *et al.* (1973) using a spectrophotometer at 520 nm wavelength.

Measurement of antioxidant enzyme activity

To extract antioxidant enzymes, fresh leaf samples (0.5 g) were collected and ground to a fine powder in a mortar by adding liquid nitrogen and then homogenized with an ice cold enzyme extraction buffer containing 0.5% polyvinylpyrrolidone (PVP), 3 mM EDTA, and 0.1 M potassium phosphate buffer (pH=7.5). The extracted samples were centrifuged for 10 min at 13500 rpm and 2-4°C and stored on ice until used. The resulting supernatants were used for enzyme analysis. CAT activity was determined according to the procedure used by Dhindsa *et al.* (1981) and SOD activity was determined as described by Beauchamp and Fridovich (1971).

Malondialdehyde (MDA)

As for H_2O_2, 0.25 g of leaf samples were ground in a mortar containing 5 ml TCA (0.1%). Leaf extracts were centrifuged at 10000 rpm for 5 min. Supernatants (250 µl) were mixed with 1 ml MDA solution containing 20% TCA and 0.5% thiobarbituric acid. The mixtures were warmed at 95°C for 30 min and then immediately cooled on ice. Sample tubes were centrifuged at 10000 rpm for 10 min. Absorption of light was measured by a spectrophotometer at 532 nm wavelength according to Heath and Packer (1969).

Hydrogen peroxide (H$_2$O$_2$)

Leaf samples (0.25 g) were ground in a mortar containing 5 ml trichloroacetic acid (TCA) (0.1%). Extracts were

centrifuged at 10000 rpm for 5 min. Supernatants (250 µl) were mixed with 250 µl phosphate buffer (100 mM) and 500 µl potassium iodide (1 M). Absorption of light was measured by a spectrophotometer at 390 nm wavelength according to Alexieva *et al.* (2001).

3. Results

With a decrease of temperature from 25°C to -7.5°C, reducing sugars increased considerably, with tall fescue showing a greater increase than common bermudagrass. The highest soluble sugar content in tall fescue was formed at -7.5°C and the highest reducing sugar content produced in common bermudagrass was detected at 0°C (Table 1). There was no significant difference between the turfgrasses for proline content. The highest proline content was observed at 0°C and the lowest proline content was seen at 25°C. The interaction of temperature and turf species showed that tall fescue at 25°C had the lowest proline content, while tall fescue at -7.5°C had the highest (Table 1). It was found that as temperature decreased from 25°C to 7.5°C, CAT activity increased.

The greatest CAT activity was observed in tall fescue at 7.5°C and the least was seen in bermudagrass at -7.5°C (Table 1). Comparison of the means showed that SOD activity in tall fescue is greater, but not significantly different from common bermudagrass. Maximum SOD activity in bermudagrass was detected at 0°C, while the minimum was found at 25°C in tall fescue. As the temperature diminished from 25°C to -7.5°C, MDA amassed continuously in the plants. MDA accumulated significantly more in common bermudagrass with the highest amount built up at -7.5°C (Table 2). H_2O_2 increased in plants as the temperature lowered to 0°C. The most H_2O_2 was produced in common bermudagrass at 0°C, whilst the lowest was observed in tall fescue at 25°C (Table 2).

4. Discussion and Conclusions

As the temperature decreased from 25°C to -7.5°C, soluble sugars and proline content increased, which tall fescue had higher amounts at -7.5°C (Table 1). A similar behavior was found in saltgrass (*Distichlis spicata* L.), centipedegrass (*Eremochloa ophiuroides* [Munro]), annual bluegrass (*Poa annua* L.) and buffalograss (*Bouteloua dactyloides* [Nutt.]) (Fry, 1993; Shahba *et al.*, 2003). Generally, one of the first reactions by these plants to counter the chilling stress of winter is a buildup of sugar (Fry, 1993; Ball *et al.*, 2002), whilst amino acids help adapt the plants to low temperature (Guy, 1990). Proline and reducing sugars serve as cryoprotectants through increasing the concentration of cell content and reducing the water potential (Ball *et al.*, 2002). Comparable results were observed in zoysiagrass (*Zoysia japonica* Steud.) and annual bluegrass (Dionne *et al.*, 2001).

Table 1 - Effects of cold stress on biochemical changes [reducing sugar, proline content, catalase (CAT) and superoxide dismutase (SOD) activity] in the two turfgrasses used in this study

Turfgrass	Temperature (°C)					Mean
	-7.5	0	+7.5	+15	+25	
Reducing sugar (mg·g⁻¹ d.w.)						
Tall fescue	176.4±60.1 a	160.8±16.3 ab	130.2±27.3 bcd	114.8±13.2 cd	104.3±22.4 d[z]	137.4 A
Bermudagrass	121.9±11.5 bcd	158.9±21.0 abc	140.9±18.7 a-d	117.3±19.8 bcd	96.5±14.7 d	127.1 A
Mean	149.2 A	159.8 A	135.5 AB	116.1 BC	100.4 C	
Proline content (µg·g⁻¹ d.w.)						
Tall fescue	35.8±3.7 a	35.1±3.4 a	27.2±2.7 b	13.7±3.1 cd	9.2±0.9 d	24.2 A
Bermudagrass	26.4±1.7 b	31.8±1.5 ab	27.3±5.1 b	17.3±4.5 c	12.7±1.3 cd	23.1 A
Mean	31.1 A	33.5 A	27.2 B	15.5 C	11.0 D	
CAT (U g·g⁻¹ d.w.)						
Tall fescue	36.3±5.4 c	46.7±6.1 ab	52.8±9.8 a	37.5±3.6 c	32.8±5.7 c	41.2 A
Bermudagrass	31.5±4.5 c	39.5±4.4 bc	47.5±2.9 ab	41.5±3.9 bc	36.9±5.8 c	39.3 A
Mean	35.4 C	43.1 B	50.1 A	39.5 BC	34.8 C	
SOD (U g·g⁻¹ d.w.)						
Tall fescue	179.6±17.6 abc	161.6±33.1 a-d	186.6±30.5 ab	126.0±19.0 de	106.6±17.0 e	152.1 A
Bermudagrass	127.3±23.1 cde	193.3±25.1 a	145.0±42.7 a-e	137.6±58.6 b-e	120.6±11.7 de	144.8 A
Mean	153.5 AB	177.5 A	165.8 AB	131.8 BC	113.6 C	

[z] In each variable, data followed by the same letters±SD (small letters for interactions and capital letters for means) are not significantly different at 5% level of probability using LSD test.

Table 2 - Effects of cold stress on malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in the two turfgrasses used in this study

Turfgrass	Temperature (°C)					Mean
	-7.5	0	+7.5	+15	+25	
MDA (µmol g⁻¹ f.w.)						
Tall fescue	7.5±1.6 cd	7.8±1.3 bc	6.8±0.8 cde	4.4±0.7 fg	3.9±0.7 g[z]	6.1 B
Bermudagrass	11.6±0.7 a	9.5±0.7 b	7.7±0.8 c	5.9±1.0 def	5.1±0.7 efg	8.0 A
Mean	9.6 A	8.6 A	7.3 B	5.1 C	4.5 C	
H_2O_2 (µmol g⁻¹ f.w.)						
Tall fescue	5.1±0.9 a	4.7±0.7 a	3.5±0.6 b	2.9±0.4 b	2.7±0.4 b	3.8 B
Bermudagrass	5.0±0.3 a	5.3±0.4 a	4.9±0.7 a	3.5±0.5 b	3.1±0.7 b	4.3 A
Mean	5.0 A	5.0 A	4.2 B	3.2 C	2.9 C	

[z] In each variable, data followed by the same letter ± SD (small letters for interactions and capital letters for means) are not significantly different at 5% level of probability using LSD test.

The increase in CAT and SOD activity found in this study is assumed to protect the cells from oxidative damage caused by cold stress as seen in other plants (Matsumura *et al*., 2002; Larkindale and Huang, 2004; Jiang *et al*., 2011). SOD converts superoxide ($O_2^{·-}$) to H_2O_2 and CAT detoxifies the latter to water and oxygen (Fuchs *et al*., 1997; Polidoros and Scandalios, 1999). Antioxidant enzymes help maintain cell homeostasis under severe low temperatures by scavenging as well as signaling, although their definite function should be further elucidated (Polle, 1997). Higher antioxidant enzyme activity in tall fescue could be attributed to better cold tolerance compared to common bermudagrass. MDA and H_2O_2 increase in the turfgrasses in this research is dependent on the cold stress received (Table 2). MDA and H_2O_2 are produced by lipid peroxidation of plants under chilling stress (Leshem, 1987;

Wise and Naylor, 1987). These two sensitive indicators are considered to point toward the extent of low temperature stress and damage inflicted to the plant (Xu *et al*., 2006). Greater amounts of these two substances in common bermudagrass compared to tall fescue could be interpreted as a greater sensitivity to and injury from low temperatures (Table 2), which is consistent with the reports in Manila grass (*Zoysia matrella* L.) (Wang *et al*., 2009).

Overall, cold stress produces large amounts of ROS which causes oxidative damage to plants through vast destruction of proteins, carbohydrates, lipids, cellular membranes, DNA and major decline of ATP reserve, and finally cell death (Dionne *et al*., 2001; Gill and Tuteja, 2010). Since ROS has multifunctional roles, it is essential for the cells to control the level of ROS tightly to avoid any oxidative injury and not to eliminate them

entirely (Sharma *et al.*, 2012). It is concluded that both turfgrasses increase reducing sugars, proline, CAT, SOD, MDA and H_2O_2 in response to lower temperatures, but tall fescue has a better defense mechanism than common bermudagrass and is more tolerant to cold stress. The results show that scavenging enzymes have a direct effect in cold season tolerance of turfgrass and improve the defense mechanism of plants, but their exact role merits further investigation.

Acknowledgements

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